BRIEF REPORT

# Successful Booster Antibody Response up to 54 Months after Single Primary Vaccination with Virosome-Formulated, Aluminum-Free Hepatitis A Vaccine

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**This study demonstrates that a booster dose of the virosome-formulated, aluminum-free hepatitis A vaccine Epaxal (Berna Biotech) is highly immunogenic in subjects who received a single primary dose of this vaccine 18–54 months earlier. There were no significant differences in geometric mean antibody titers (GMTs) among subjects who received the booster dose 18–29 months (GMT, 2330 mIU/mL), 30–41 months (GMT, 2395 mIU/mL), or 42–54 months (GMT, 2432 mIU/mL) after primary vaccination, indicating that delays in the administration of booster vaccination do not lead to a loss of immunogenicity.**

Hepatitis A is an acute, usually self-limiting infection caused by hepatitis A virus (HAV). In areas of high endemicity, such as Africa and parts of Asia and Latin America, infection with HAV primarily occurs during childhood, which provides lifelong immunity against the disease. However, adult travelers from countries of low HAV endemicity who visit regions of high endemicity are at risk of acquiring clinically symptomatic infection, and it is therefore recommended that they receive vaccination against HAV infection [1–3]. To obtain long-lasting protection against HAV, 2 doses of vaccine, administered 6–18 months apart, are recommended [3]. In practice, however, many travelers do not return within 18 months for booster vaccination. Therefore, it is important to know how long the booster vaccination can be delayed without loss of seroprotection.

Epaxal (Berna Biotech), the only aluminum-free anti-HAV vaccine available, is based on formalin-inactivated HAV, which is attached to the surface of special liposomes (virosomes). These virosomes replace aluminum hydroxide as the adjuvant principle. The virosomes contain the hemagglutinin antigen from the influenza A virus, which physiologically enhances the immune response to inactivated HAV [4, 5]. A single injection of Epaxal has been shown to be safe, well tolerated, and highly immunogenic [5–7]. The aim of this study was to investigate the immunogenicity of Epaxal given as a booster dose >18 months after primary vaccination with the same vaccine in healthy adult travelers.

***Patients, materials, and methods.*** This open-label, noncomparative, single-center study assessed the immunogenicity and tolerability of a single booster dose of Epaxal in 115 healthy men and women (age, ⩾18 years) who had undergone primary vaccination with a single dose of Epaxal ⩾18 months earlier.

Subjects who had previously undergone both primary and booster vaccination against HAV infection; who had an acute febrile illness, known immunodeficiency, or history of allergy or atopy; who had participated in another study; or who were pregnant or lactating were excluded from the study. Concomitant treatment with any immunosuppressive drug was also prohibited, as was receipt of a blood transfusion, immunoglobulins, or any investigational drug during the 3 months before the study. The study was approved by the Ethics Committee of Basel (Basel, Switzerland) and was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent before entry into the study.

Eligible subjects were vaccinated with a single dose of Epaxal, which was injected intramuscularly into the deltoid muscle of the left or right upper arm. The vaccine was supplied in ready-to-use syringes containing 0.5 mL of vaccine, which included ⩾500 RIA units of HAV antigen, 10 $\mu$g of influenza A (H1N1) hemagglutinin, and 350 $\mu$g of phospholipid. The inactivated, whole HAV contained in Epaxal is derived from the HAV strain RG-SB, purified from MRC-5 human diploid cell cultures, and inactivated in formalin [4, 5].

Blood samples were obtained at baseline (before injection [day 1]) and ~1 month later (days 26–46) for measurement of serum HAV antibody titers by EIA (Enzymun; Boehringer Mannheim) at the University Children's Hospital (Basel). Titers were expressed as HAV antibody concentrations in mIU/mL.

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Solicited data on adverse events and spontaneously reported adverse events were documented and assessed by the investigator at each clinic visit (i.e., before vaccination [baseline] and ~1 month later). Subjects completed a checklist for specific local and systemic reactions, including pain/tenderness, hardness, swelling/tumefaction, redness (diameter, >5 mm), headache, fatigue, arthralgia, loss of appetite, and nausea, and recorded their axillary temperature over a 4-day period after injection. The intensity of each adverse event was assessed using a 4-point rating scale, as follows: 0 for "none," 1 for "mild" (i.e., event did not interfere with daily activities), 2 for "moderate" (i.e., event interfered with daily activities), and 3 for "severe" (i.e., event prohibited normal daily activities).

Subjects were evaluated if they had no evidence of prior HAV infection (i.e., an anti-HAV titer of <7000 mIU/mL) before receiving the booster dose of Epaxal, and subjects had HAV antibody titers measured before and after receipt of this booster dose. Seroprotection was defined as an HAV antibody titer of ⩾20 mIU/mL. In exploratory analyses, an HAV antibody titer of ⩾10 mIU/mL was also considered seroprotective, which is consistent with studies of another inactivated HAV vaccine, Vaqta (Merck) [8]. Geometric mean titers (GMTs) and seroprotection rates were summarized according to time interval after primary vaccination. The effect of the time interval since primary vaccination on the HAV antibody response was analyzed using logistic regression analysis. All subjects who received the study vaccine and completed the follow-up were evaluated for safety.

***Results.*** One hundred seventeen subjects were enrolled, of whom 115 (53 men and 62 women) completed the study and were evaluable for safety. The mean age was 44.8 years (range, 20–70 years). One subject was withdrawn from the study because of a screening error, and 1 subject failed to attend a follow-up assessment.

Ninety-seven subjects were evaluated for immunogenicity. Eighteen subjects were excluded from efficacy evaluation: 16 subjects had evidence of prior HAV infection (anti-HAV titer on day 1, 11,559–70,000 mIU/mL), the serum sample obtained on day 1 was missing for 1 subject, and the date of primary vaccination could not be confirmed for 1 subject.

Before booster vaccination, 89% and 67% of 36 subjects who received the booster dose at 18–29 months had HAV antibody titers of ⩾10 or ⩾20 mIU/mL, respectively. These HAV antibody titers were found in 91% and 77% of 34 subjects, respectively, who received the booster dose at 30–41 months and in 85% and 70% of 27 subjects, respectively, who received the booster dose at 42–54 months. One month after booster vaccination, the GMT was 2385 mIU/mL for the whole group, and seroprotection was achieved in 100% of subjects. Logistic regression analysis showed that there were no statistically significant differences in GMT between subjects who received the booster dose 18–29 months (GMT, 2330 mIU/mL; 95% CI, 1538–3529 mIU/mL), 30–41 months (GMT, 2395 mIU/mL; 95% CI, 1563–3672 mIU/mL), or 42–54 months (GMT, 2432 mIU/mL; 95% CI, 1506–3928 mIU/mL) after the first dose—that is, the immune response was independent of the time since primary vaccination (figure 1).

The antibody titers of 1 vaccinee (see footnote *a* in figure 1) require comment. A 31-year-old man responded to the booster dose with a titer of 31 mIU/mL only. Before receiving the booster dose (36 months after the primary vaccination), the antibody titer had decreased to 8 mIU/mL. A thorough clinical and laboratory evaluation did not reveal any abnormalities. A third dose was given 17 months after the booster, and, 6 weeks after the third dose, the titer was 261 mIU/mL. At the same time, an anti–hepatitis B antibody titer of >1500 mIU/mL was recorded after receipt of a third dose of hepatitis B vaccine. No factor (e.g., age or whether the subject smoked or was overweight) that could explain this low response to anti-HAV antigen was found in this apparently healthy subject.

Booster vaccination was well tolerated by all subjects. No serious systemic or local adverse events were reported. The vast majority of local adverse events were of mild intensity and resolved later the same day. Local adverse events were reported by 31% of subjects. The most common local events were pain or tenderness (23% of subjects) and induration (17% of subjects). Systemic adverse events were reported by 46% of subjects. However, a considerable proportion (24%) of these subjects reported the same adverse symptoms before booster vaccination upon solicited questioning. Only 1 subject had an increased axillary temperature (⩾37.5°C). The most frequently

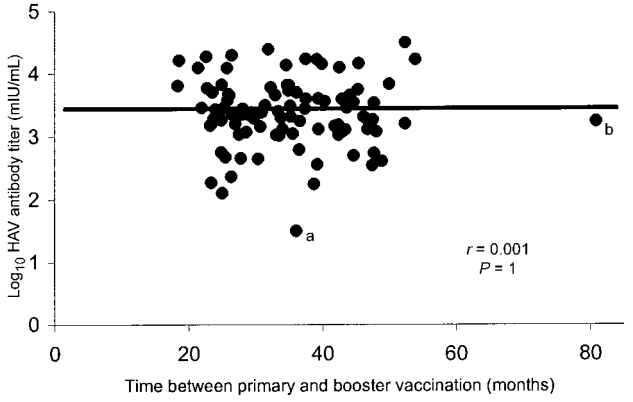


**Figure 1.** Scatterplot of log-transformed hepatitis A virus (HAV) antibody titers after booster vaccination, as a function of the time between primary and booster vaccination. [a]Data for a 31-year-old man who responded to the booster dose with a titer of 31 mIU/mL only (see Results). [b]Data for a subject who participated in an earlier trial of primary vaccination 81 months before booster vaccination.

reported systemic symptoms at baseline and after vaccination were fatigue, headache, and arthralgia.

***Discussion.*** The results of this study demonstrate that a delay of up to 54 months between primary vaccination and receipt of a booster dose does not influence the immune response to Epaxal. Seroprotection rates after primary vaccination were similar in subjects who received the booster dose after 18–29, 30–41, or 42–54 months, suggesting that the rate of decline in antibody response after priming did not increase over time. A single booster dose of Epaxal induced a strong antibody response in all groups, including subjects with HAV antibody titers of <10 mIU/mL at enrollment, which suggests that an anamnestic immunological response was effectively developed [9]. The booster vaccination with Epaxal was well tolerated in all groups. The observation that the interval between administration of primary and booster vaccinations did not have any influence on the immune response to the booster dose are consistent with results from similar studies using an aluminum-adsorbed vaccine [10, 11].

The results of this study are of practical relevance for clinicians in travel medicine, because they demonstrate that delays in the administration of booster vaccination do not lead to loss of immunogenicity. Many travelers return for booster vaccination much later than the recommended 12 months, often before new travel. The antibody response found in the present study indicates that booster doses are highly immunogenic for up to 4–5 years after primary vaccination.

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